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Determinants of Plasma Copeptin in Newborns at Birth and During Postnatal Adaptation

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1. Summary

Background. Arginine vasopressin (AVP) is a key hormone in regulating blood pressure and body water balance, but despite modern procedures remains difficult to measure. Copeptin, the carboxyl-terminus portion of the AVP precursor, is much more stable and presents a reliable alternative to measuring the secretion of AVP. Copeptin has been studied to great extent in adults and is emerging as a useful diagnostic and prognostic biomarker for a variety of diseases. As of now there has been no research on copeptin in pediatric medicine. The aim of this study was to assess the normal basal values of plasma copeptin in infants in the first days of life and to investigate influencing factors at birth and during early postnatal adaptation.

Methods. A prospective cross-sectional study was carried out at Zurich University Hospital and included 177 infants born between 32 and 41 weeks of gestation. Blood samples were obtained from the umbilical artery and the umbilical vein at birth and again from venous puncture at day 3 of life. The collected samples were prepared and then analyzed for their copeptin content using the CT-proAVP luminescence immunoassay (B.R.A.H.M.S Company, Hennigsdorf, Germany).

Results. One hundred seventeen paired blood samples were collected from umbilical arteries and veins, along with an additional twenty-six unpaired venous cord blood samples. Copeptin in the umbilical artery was consistently higher than in the umbilical vein (median 18.2 [range 2.38-5000] pmol/L vs. 10.2 [1.87-5000] pmol/L) and there was a strong relationship between these values (Spearman's rank correlation coefficient (R_S)=0.825, $p<0.001$). Overall, copeptin levels at birth were much higher after vaginal delivery compared to after caesarean section. Furthermore,

the copeptin concentrations in arterial and venous umbilical cord plasma correlated significantly with both the pH value (R_s=-0.639, R_s=-0.578, both p<0.001, respectively) and the base excess (R_s=-0.645, $R_{s=}$-0.638, both p<0.001, respectively) in the umbilical artery.

One hundred and two venous blood samples were collected at day 3 of life (of which sixty-eight having a paired venous sample from birth). Average plasma copeptin levels at day 3 of life were similar to those at birth, but the distribution of values was much smaller in comparison (median 13.5 [range 4.63-169] pmol/L). Finally, plasma copeptin concentrations at day 3 of life were directly correlated to the maximal postnatal weight loss experienced by the infant (R_s=0.438, p<0.001), which in turn was inversely correlated to copeptin concentrations at birth in the umbilical artery and vein (R_s=-0.309, R_s=-0.289, both p=0.001, respectively).

Conclusion. Exceptionally high copeptin values were related to vaginal delivery and birth acidosis in healthy newborns. Copeptin release at birth is therefore likely brought on by birth stress. Plasma copeptin concentrations at day 3 of life provided insight to the actual water balance of the infant, which was less negative in infants who experienced a larger copeptin release at birth. The results from this study put forward important functions of AVP in primary and secondary adaptation in newborns and attest a role of copeptin measurement at birth in detecting fetal distress, particularly perinatal asphyxia.

2. Background

Vasopressin: Biochemical Properties and Function

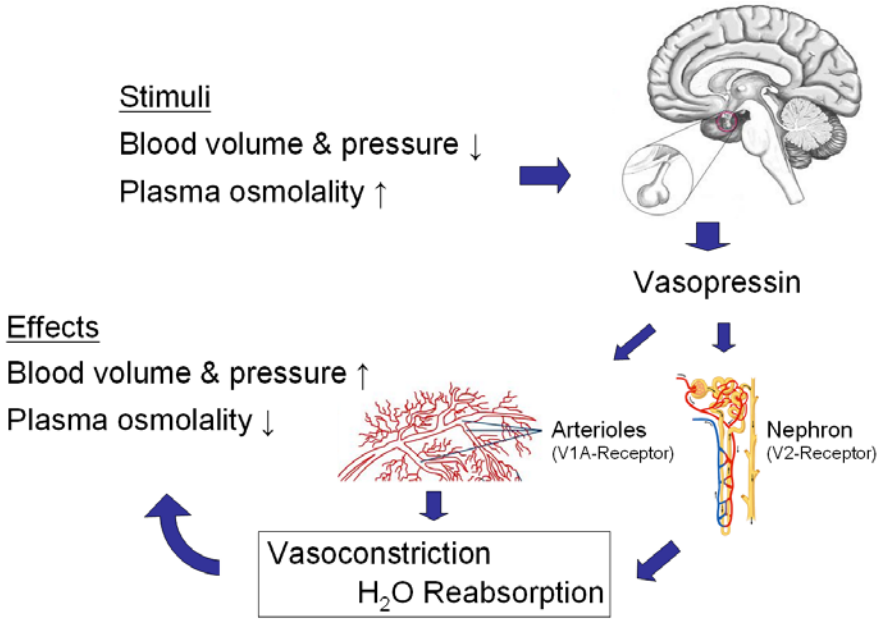
Arginine vasopressin (AVP), also referred to as antidiuretic hormone (ADH), is a nonapeptide hormone produced mainly by AVP-specific magnicellular neurons in the supraoptic nuclei (SON) and the paraventricular nuclei (PVN) of the hypothalamus, whose axons project down to make up the posterior pituitary gland [1]. Another group of AVP-producing neurons is located in the suprachiasmatic nucleus [2]. AVP derives from a larger precursor protein, pre-provasopressin (pre-proAVP), which consists of four segments: a signal peptide, AVP itself, neurophysin II and copeptin [3]. After translation, pre-proAVP is packaged into vesicles and transported to the neurohypophysis axon endings [4], undergoing various enzymatic modifications on the way before finally being cleaved into its individual components [3]. Appropriate stimulation of vasopressinergic neurons creates an action potential, which provokes a fusion of the neurosecretory vesicles with the cell membrane of the axon endings [5], releasing the peptides in equimolar concentrations [6] into the bloodstream where they circulate independently from one another [3]. The same stimulation also increases the rate of transcription [7] and upgrades the axonal transport of the neurosecretory vesicles [4]. The elimination of AVP from circulation is ensured mainly by renal but also hepatic clearance [8,9].

A variety of stimuli, mainly changes in plasma osmolality and in the pressure-volume system, account for AVP release (Figure 1). Osmotic stimuli are perceived by osmoreceptors in different regions of the brain [10]. There is a positive linear relationship between plasma osmolality and basal plasma AVP in healthy persons [11]. The osmoreceptors are under a constant mild stimulation but are very sensitive to change, where even the slightest increase in plasma osmolality causes an increase

in plasma AVP levels [11]. Changes in blood pressure or volume are detected by high-pressure arterial baroreceptors and low-pressure atrial volume receptors [12]. These changes, however, must be of a certain magnitude in order to bring out an AVP release: only a sufficient decrease in either provokes an increase in plasma AVP [11,12]. Other relevant stimuli for AVP release are nausea and hypoglycemia [13,14].

The hormone operates in the periphery by binding to either one of three G protein-coupled AVP receptors on target cells: the V1a, V1b and V2 receptors [15]. V2 receptors are located predominantly on the basolateral membrane of cells of connecting tubules and collecting ducts in the nephron. AVP binding results in the insertion of previously stored aquaporin 2 water channels into the apical cell membrane and in an increased aquaporin 2 synthesis, allowing greater water reabsorption by the kidney [15] (Figure 1). V1a receptors are found primarily on smooth muscle cells of blood vessels and provoke vasoconstriction from higher plasma AVP concentrations [15] (Figure 1). V1b receptors found on the corticotropic cells of the anterior pituitary gland are involved in the secretion of adrenocorticotropic hormone [15] and thus in the hypothalamic-pituitary-adrenal axis stress response [16,17]. Interestingly, AVP receptors have also been found in the brain [18,19]. This direct presence of AVP in the central nervous system is likely relevant for many central functions, such as social behavior, learning and memory processes, and central temperature and cardiovascular regulation [20].

Figure 1: Overview on vasopressin regulation



AVP is involved in numerous pathologies. Diabetes insipidus (DI), the clinical presentation of a reduced effect of AVP, is brought on either by decreased AVP production by the hypothalamus (central DI) or by AVP resistance in the kidney (nephrogenic DI). Its main feature is an excessive and diluted urine production [21]. The Syndrome of Inappropriate Antidiuresis (SIADH) is characterized by an increased, non-physiological AVP activity and results in hyponatremia and the production of inappropriately concentrated urine [21]. In the presence of early vasodilatory septic as well as cardiogenic shock plasma AVP levels are higher than normal [22,23], and subsequent AVP administration improves hemodynamic parameters in these shock states [24,25]. Today, AVP is also considered a reasonable first-line vasopressor in advanced cardiac life support, although the benefit remains questionable [26,27]. Lastly, clinical observations indicate an involvement of AVP in the pathogenesis of heart failure and liver disease [28,29].

Vasopressin in Children

During human ontogenesis, AVP immunoreactive cells can be detected in the SON and the PVN at as early as eighteen weeks of gestation [30]. The expression of AVP mRNA begins in the second trimester and average levels of the hormone in the hypothalamus and pituitary gland then increase with fetal age until birth [30,31]. Furthermore, there is evidence from animal studies that AVP expression through osmotic stimulation is already functional during the fetal period [32,33]. At birth, human AVP synthesis and secretion is intact. Average AVP concentrations in umbilical cord blood plasma are greater than average plasma concentrations measured in adults, and depending on the mode of delivery can reach very high levels [34,35]. "High AVP concentrations in cord blood are associated with higher blood pressure and lower skin temperature, indicating peripheral vasoconstriction" and a fully functional hormone [36]. Plasma AVP levels then decline after birth and normal basal AVP in healthy children above one year of age does not differ from healthy adults and similarly does not correlate with age [37,38]. The necessary osmotic stimuli for secretion are the same: plasma AVP concentrations in children correlate significantly with plasma and urine osmolality [37].

Just as in adults, AVP is secondarily involved in the pediatric endocrine response to several hemodynamic disorders. Circulating AVP levels are elevated in septic children [39]. In managing refractory hypotension due to either septic or vasodilatory and cardiogenic shock after heart surgery, AVP is an effectively proven vasopressor [40–42]. Its current role in pediatric cardiac arrest, however, is still undetermined [43].

Vasopressin Measurement

Circulating AVP is generally measured with a radioimmunoassay involving specific AVP antiserum [11]. Precise measurement is ensured by the high sensitivity and

specificity of this test; however, it is challenging to obtain accurate results. In vivo, AVP is quickly degraded resulting in a half-life of a few minutes [8] and remains unstable even once collected in tubes with or without a matrix, for example EDTA [11]. This necessitates fast, yet impractical measurement. Although it does not bind to plasma proteins [8], circulating AVP does indeed bind to platelets [44]. Exact sample preparation is therefore required to "avoid contamination of plasma with platelet-bound AVP" and otherwise falsely representative levels [38]. Finally, test samples must be modified before analysis with a series of complicated steps [11,45].

For these reasons routine measurement of AVP has little clinical relevance, even for diagnosing AVP dysfunction. Diabetes insipidus is usually diagnosed by performing a water deprivation test in which urine and plasma osmolality are measured rather than AVP; the latter is reserved for when there is diagnostic doubt [21]. For SIADH, elevated blood AVP concentrations are not essential diagnostic criteria [21].

Copeptin: An Adequate Marker for Vasopressin

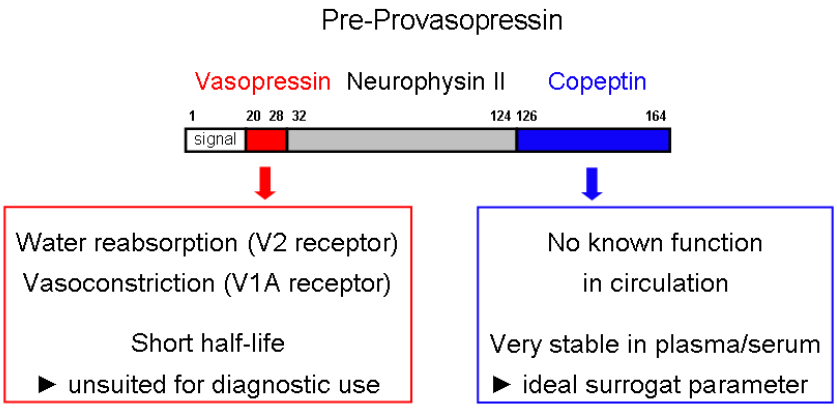
As introduced above, copeptin is the carboxyl-terminus of pre-proAVP and is released together with AVP in equimolar concentrations (Figure 2). It is unclear whether it has a concrete function in the periphery, but it is probably involved in pre-hormone assembly [46]. Unlike AVP, copeptin measurement is less troublesome. A highly sensitive chemiluminescence sandwich immunoassay for copeptin allows precise measurement, having many advantages over the AVP radioimmunoassay [45]. Results are available after only a few hours, no preanalytical procedures are required and sample quantities of as little as 50 µL plasma suffice [47]. Contrary to AVP, copeptin is considerably stable once collected, especially in EDTA-plasma [45]. Sample storage here is permitted for up to 14 days at room temperature with virtually no

change in the resulting mean measured value compared to the original value [45]. The same applies to storage at 4° C [45].

Seeing that copeptin is produced and released along with AVP in equimolar concentrations, measurement of circulating copeptin presents a less complicated yet reliable means of indirectly measuring AVP (Figure 2). There is a documented significant correlation between normal plasma copeptin and normal plasma AVP in healthy adults [45]. Moreover, copeptin mirrors AVP secretion in the presence of its physiological stimuli. Plasma copeptin levels correlate with plasma osmolality in healthy subjects [48], and water intake and deprivation result in a decrease and increase in copeptin, respectively [45,48]. As expected, copeptin also reacts to changes in blood volume and pressure. In an animal model, baboons exposed to bleeding responded with increases in their plasma copeptin concentrations according to the degree of blood loss and hypotension; after reperfusion, the copeptin levels then slowly declined [49]. In man, a similar response can be observed: a decrease in mean arterial blood pressure due to septic shock leads to proportionally increased plasma copeptin concentrations [49].

The ability to mirror AVP secretion is not lost under pathological conditions. With the case of primary AVP dysfunction, copeptin levels are pathologically low in patients with central DI [50]. Moreover, in one study, plasma copeptin concentrations in adults presenting with Systemic inflammatory response syndrome (SIRS), sepsis or after cardiac surgery were investigated and compared with their corresponding plasma AVP concentrations: the resulting mean copeptin values were consistently elevated, and here too a significant correlation between copeptin and AVP was observed [51].

Figure 2: Overview on vasopressin and copeptin synthesis and function



Copeptin: Role in Disease and as a Biomarker

As previously mentioned, critical illness is characterized by significantly elevated plasma copeptin concentrations [51], which increase with ascending degree of illness [49]. Furthermore, it has been shown that average copeptin values are higher in nonsurvivors of sepsis or septic shock than in survivors [49]. Myocardial infarction is also associated with elevated plasma copeptin levels [52]. According to one study, the combination of copeptin and standard troponin T measurement improved early diagnosis after the onset of typical symptoms of acute coronary syndrome [53]. In addition, copeptin is a possible prognostic biomarker with regards to mortality in the days following an acute myocardial infarction as well as for the imminent development of heart failure [52,54]. Plasma copeptin is also elevated in pulmonary disease, and is being investigated as a possible prognostic biomarker for clinical outcome in affected patients [55,56]. Lastly, there is an association between copeptin and stroke. In the case of acute intracerebral hemorrhage, copeptin again correlates with the severity of illness and further acts as a prognostic biomarker: patients with higher plasma copeptin concentrations on admission have a higher risk of early mortality and/or unfavorable functional outcome [57]. Similarly, copeptin appears to be

an “independent predictor of functional outcome and death” in patients with acute ischemic stroke [58].

The use of this novel and seemingly valuable biomarker in pediatric medicine has been hindered thus far by the lack of data available in this field. This study aimed to alter this dilemma.

3. Methods & Materials

A prospective cross-sectional study was conducted from July 2009 to September 2009 in the maternity and neonatology wards of Zurich University Hospital and was approved by the ethics committee of the hospital (Kantonale Ethikkommission Zürich, KEK 08/09). The participating expectant mothers gave written informed consent upon admission.

Details of pregnancy (presence or absence of preeclampsia, diabetes, infection, preterm labor or administration of betamethasone for fetal lung maturation), delivery (umbilical artery pH, umbilical artery base excess and hematocrit; amount of maternal blood loss) and the infants' birth characteristics (gestational age, birth weight, APGAR scores at 5 and 10 minutes) were collected from the charts. Data on the infants' health after birth, whether the infants were exclusively breast fed, additionally fed with formula or given intravenous fluid, and the daily weight control were recorded by the staff on the maternity or neonatology wards.

Up to a maximum of three blood samples was collected per infant. The first and second of the three samples were drawn immediately after birth, one from the umbilical cord artery and one from the umbilical cord vein. The last of the three samples contained venous blood drawn from the back of the hand or foot after 64-96 hours of life, referred to as day 3 of life.

Approximately 0.3-0.5 ml of blood was drawn per sample and held in EDTA tubes. These were immediately placed at 4° C and stored for a maximum of four hours. Centrifugation was done at 3220 G for two minutes, after which the supernatant containing EDTA-plasma was displaced into a new EDTA tube that was promptly stored at -28° C until analysis.

Copeptin measurement was performed using the CT-proAVP luminescence immunoassay, which was provided and realized by the B.R.A.H.M.S Company (Hennigsdorf, Germany). Briefly, three peptides representing three distinct sequences positioned on copeptin were chemically synthesized [45]. Antibodies directed against these peptides were created by immunizing sheep with each individual peptide and obtaining their antisera [45]. One purified antibody was labeled with MACN-Akridinium-NHS-Ester and diluted into assay buffer to produce the tracer [45]. Polystyrene tubes were coated with one of the other purified antibodies [45]. The last of the three antibodies was diluted in horse serum and used as a calibrator [45]. Fifty microliters of plasma samples/calibrators were incubated with 200 μL of tracer "in the coated tubes under agitation for two hours at room temperature", the tubes were consequently "washed four times…and the bound chemiluminescence was measured for one second per tube with a luminometer" [45]. The lower detection limit was determined at 1.7 pmol/L and the upper detection limit at 5000 pmol/L [45].

Statistical analysis was performed with the SPSS Statistics program, PASW 18.0 (SPSS inc., Chicago, Ill.). Specifically, strictly non-parametric tests were applied; comparisons of non-parametric data were done using the Wilcoxon signed-rank test for paired samples, and the Mann-Whitney U and Kruskal-Wallis tests for independent groups. Correlation was determined with the Spearman's rank correlation test. The level of significance was set at $p<0.05$.

4. Results

A total of 362 samples were collected from 177 infants. One hundred seventeen blood samples were collected from the umbilical artery, all of which had a corresponding paired sample from the umbilical vein. An additional 26 venous cord blood samples were further collected. One hundred and two venous blood samples were collected at day 3 of life, sixty-eight of which having a corresponding venous umbilical cord blood sample from birth.

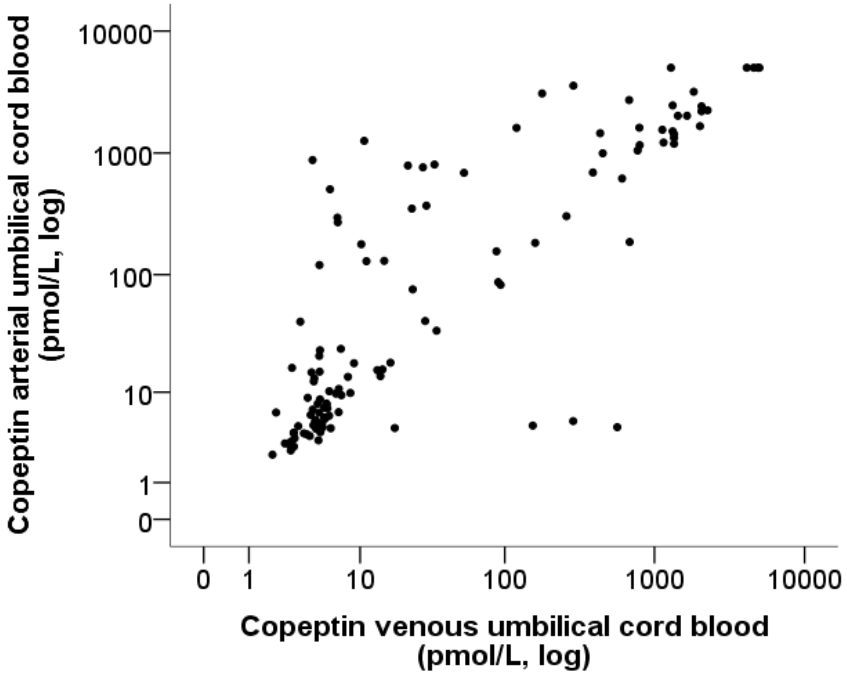
Of the 177 infants studied, 141 (80%) were term (37-41 completed weeks of gestation), 21 (12%) were near-term (35 or 36 weeks), and 15 (8%) had a gestational age of 32-34 weeks. Twenty-four (14%) infants were twins and three (2%) were triplets. Sixty-two (35%) of the 177 infants were delivered vaginally, including 17 (10%) cases requiring instrumental support; 115 (65%) were delivered by caesarean section (C-section), including 40 (23%) cases of C-section with prior onset of labor after the presentation of acute fetal or maternal distress (also referred to as secondary C-section). The percentage of deliveries by C-section was significantly higher in preterm infants (31 of 36, 86%) than in term infants (83 of 141, 59%, p=0.003). For details see also Table 1.

Table 1: Selected characteristics of mothers and their infants

Characteristics	Venous plasma umbilical cord (n = 143) n (%)	Arterial plasma umbilical cord (n = 117) n (%)	Venous plasma day 3 (n = 102) n (%)
Infant sex			
Male	78 (55)	69 (59)	60 (59)
Infant birth weight (g)			
< 2000	11 (8)	6 (5)	8 (8)
2000 - 3000	47 (32)	38 (31)	26 (26)
3001 - 4000	77 (54)	66 (56)	61 (61)
> 4000	8 (6)	7 (6)	7 (7)
Infant gestational age at birth (completed weeks)			
Preterm 32 - 36	32 (22)	26 (22)	20 (20)
Term 37 - 41	111 (78)	91 (78)	82 (80)
Mode of delivery			
Caesarean section elective	74 (52)	63 (54)	42 (42)
Caesarean section with labor	26 (18)	23 (20)	21 (21)
Spontaneous vaginal	30 (21)	23 (20)	28 (28)
Instrumental vaginal	13 (9)	8 (7)	11 (11)

The median copeptin concentration at birth was 18.2 pmol/L (range 2.38-5000 pmol/L) in arterial cord blood and 10.2 pmol/L (range 1.87-5000 pmol/L) in venous cord blood. At day 3 of life, the median venous copeptin concentration was 13.5 pmol/L (range 4.63-169 pmol/L). The difference in the average values of the paired umbilical cord samples was statistically significant ($p<0.001$). Furthermore, these paired sets of values were very strongly and significantly correlated with one another ($R_S=0.825$, $p<0.001$); when copeptin in the arterial cord blood sample increased, it regularly increased in the corresponding venous cord blood sample (Figure 3). No relationship could be determined with regards to the paired venous samples from birth and day 3 of life.

Figure 3: Correlation of copeptin concentrations in paired arterial and venous umbilical cord blood samples

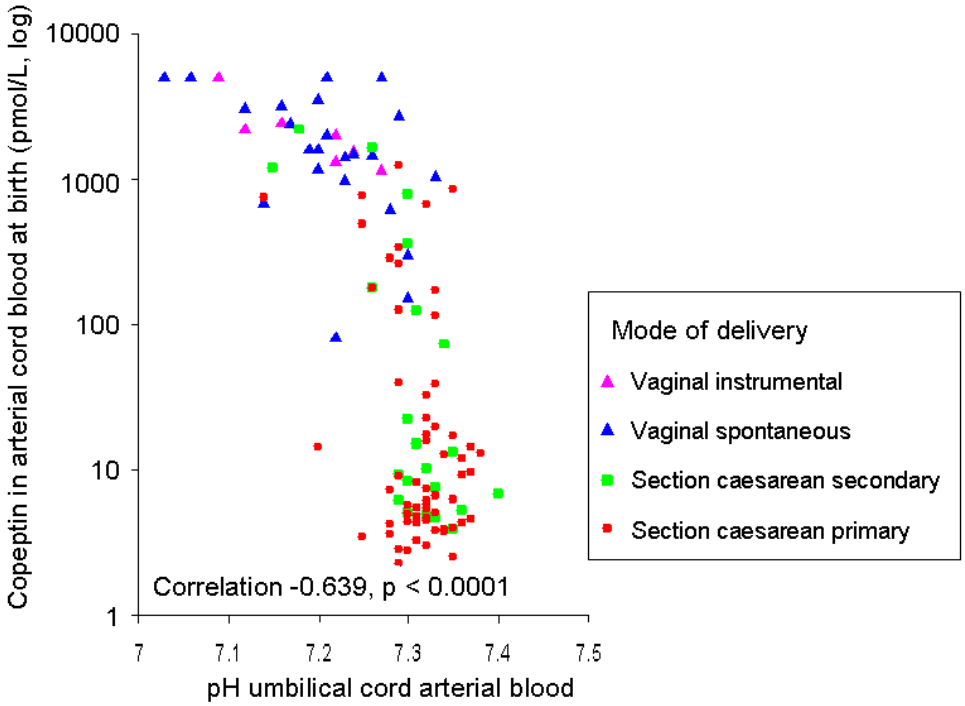


There was no significant gender difference in the average copeptin values in any of the three samples. As to gestational age there was a significant positive correlation with copeptin in both the umbilical artery and vein (R_s=0.377, R_s=0.416, both p<0.001, respectively), but none with copeptin at day 3 of life. Birth weight correlated weakly with copeptin concentrations in venous cord blood only (R=0.183, p=0.029). There was no correlation between the APGAR scores at 5 and 10 minutes or the hematocrit in umbilical cord blood and the copeptin concentrations in any plasma samples.

There was a very significant inverse correlation between the pH value measured in umbilical artery blood and the copeptin concentrations in both arterial and venous cord blood (R_s=-0.639, R_s=-0.578, both p<0.001, respectively). At physiological pH values, copeptin levels remain under a certain threshold and then tend to increase as

the pH decreases (Figure 4). Accordingly, there was also a very significant inverse correlation between umbilical artery base excess and the copeptin concentrations in both arterial and venous cord blood (R_S=-0.645, R_S=-0.638, both p<0.001, respectively). Contrarily, neither base excess nor pH correlated with copeptin concentrations measured in venous plasma at day 3 of life.

Figure 4: Copeptin in umbilical artery blood in relation to arterial pH and delivery mode



As to the mode of delivery, spontaneous and assisted vaginal deliveries were associated with much higher median copeptin concentrations in both arterial and venous cord plasma. In comparison, C-section, with or without prior onset of labor, was associated with lower copeptin values at birth (Table 2). The median copeptin concentrations at day 3 of life were similar in all groups regardless of delivery mode.

Table 2: Copeptin intervals in arterial (a) and venous (v) umbilical cord plasma				
Mode of delivery	n a / v	Median copeptin pmol/L a / v	95% reference interval, pmol/L a / v	P[a] a / v
Caesarean elective	63 / 73	8 / 5	3-907 / 5-504	
Caesarean with labor	23 / 27	14 / 11	4-2240 / 2-2260	< 0.01 / < 0.01
Vaginal spontaneous	23 / 30	1610 / 644	82-5000 / 6-5000	< 0.001 / < 0.001
Vaginal instrumental	8 / 13	1786 / 1324	1786-5000 / 90-4900	< 0.001 / < 0.001

[a] Significance between caesarean primary and with each other mode of delivery; a, arterial; v, venous

Concerning pregnancy and labor, there was a significant (p=0.003, p=0.001, respectively) difference in both arterial and venous umbilical cord plasma copeptin concentrations in the group of expectant mothers who had experienced a failure in progress of labor compared to those who did not. In the first group, average copeptin levels tended to be higher. The same was observed in the groups who had received synthetic oxytocin or prostaglandin E1 analogue administration during labor and in the group in which an epidural anesthesia was performed (p<0.001, throughout). In all of these subgroups, however, no significant difference was found in copeptin at day 3 of life.

Finally, there was a very significant inverse correlation between maximal weight loss during postnatal adaptation and the copeptin concentrations in arterial as well as venous umbilical cord plasma at birth (R_s=-0.309, R_s=-0.289, both p=0.001, respectively). On the other hand, maximal postnatal weight loss correlated directly with the copeptin concentration in venous plasma drawn at day 3 of life (R_s=0.438, p<0.001). These correlations are illustrated in Figures 5 and 6.

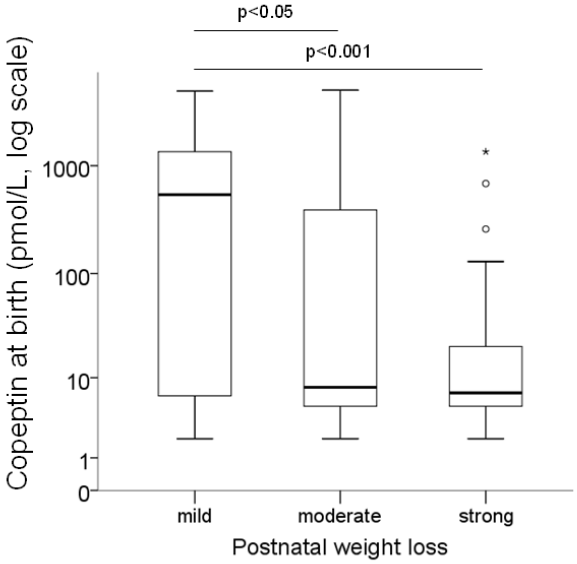


Figure 5: Correlation of copeptin in venous umbilical cord plasma with maximal postnatal weight loss. Maximal postnatal weight loss is grouped in mild (2-5%, n=33), moderate (6-7%, n=51), and strong loss (8-12%, n=53). Data are presented as box (interquartile range) and whisker (5-95 % range) plots.

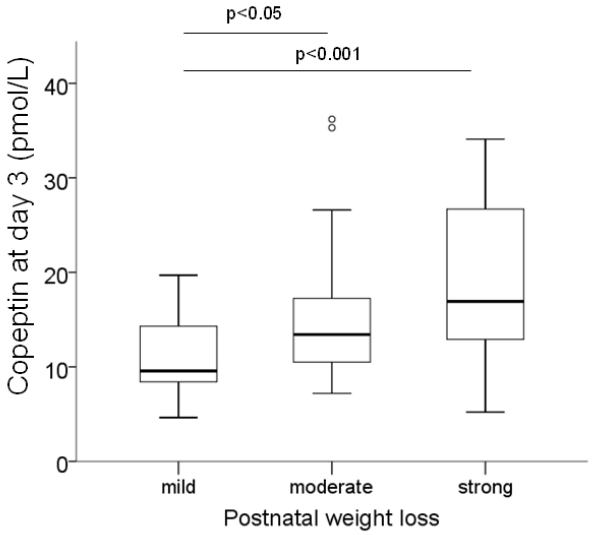


Figure 6: Correlation of copeptin in venous plasma at day 3 with maximal postnatal weight loss. Maximal postnatal weight loss is grouped in mild (2-5%, n=29), moderate (6-7%, n=36), and strong loss (8-12%, n=37). Data are presented as box (interquartile range) and whisker (5-95 % range) plots.

5. Discussion

In this study copeptin was measured for the first time in newborn infants. The aim of this study was to establish copeptin reference values for this group and to determine the effects of various perinatal factors on copeptin concentrations at birth and at day 3 of life. The study was performed in a non hypothesis-driven manner.

Copeptin in arterial umbilical cord plasma was consistently higher than in venous umbilical cord plasma. This supports the notion that the measured copeptin originates from the fetus and not from the mother. It was reported for anencephalic newborns that they did not have circulating AVP [59], clearly demonstrating that the human placental barrier is tight for AVP [60].

Interestingly, there was a very large distribution of copeptin values at birth with maximum concentrations reaching the upper detection limit of the immunoassay (5000 pmol/L) in both sets of cord blood samples. This was not once the case in any of the plasma samples collected at day 3 of life, where the highest measured copeptin value amounted to 169 pmol/L. Hence, even maximum copeptin concentrations present at birth all returned to normal low levels by day 3 of life.

The observations made in this study were in accordance with previous findings made for AVP, which at birth and especially after vaginal delivery can be present at very high plasma concentrations that decrease shortly afterwards to normal low levels [35–37]. Therefore, copeptin appears to accurately mirror AVP activity also at birth. One limitation to this study, however, was that AVP was not measured to prove any direct correlation between AVP and copeptin during early neonatal life.

Birth is a stressful event for both mother and child, and AVP is known to be involved in the fetal stress response [36,61]. In this study the same was observed for copeptin. Overall, the average copeptin values in umbilical cord plasma of neonates born

without any birth stress (elective C-section group) were within the range of normal copeptin values found in healthy adults (median 4.2 pmol/L, range 1-13.8 pmol/L [45]). In contrast, in the presence of any birth stress, umbilical cord copeptin levels rose exceptionally. Vaginal delivery especially was associated with much higher birth copeptin levels, namely a 200-fold increase, as compared to C-section delivery, indicating a clear link to birth stress. This was furthermore supported by the strong relationships found between cord blood copeptin and established blood stress markers such as the pH value and base excess in umbilical artery blood. Average copeptin values at birth were even lower after elective C-section compared to after C-section with prior onset of labor, hence the presence of contractions is important for copeptin release. It is well known that uterine contractions cause a transient decrease in maternal-fetal blood flow and subsequently a transient hypoxia in the fetus. The here presented data on copeptin release fitted to the previous findings that hypoxic stress triggers AVP release [62,63].

Neither mode of delivery nor birth acidosis influenced copeptin concentrations in venous plasma drawn at day 3 of life. Moreover, there was no correlation between copeptin concentrations at birth and those at day 3 of life. These findings can be interpreted in two ways. First, once the posterior pituitary releases all of its stored AVP/copeptin, it requires a bit of time to replenish itself. This may be the case in infants with exceptionally high copeptin concentrations at birth and copeptin concentrations below the average at day 3 of life. In contrast to this hypothetical conclusion, a second interpretation is based on additional findings. The direct correlation between maximal postnatal weight loss and plasma copeptin concentrations at day 3 of life illustrated an appropriate AVP response to negative water balance, whereas infants with elevated copeptin concentrations at birth subsequently had a milder postnatal weight loss. Therefore, a second plausible

conclusion is that AVP/copeptin can be released in extremely high amounts at birth and still the AVP system is able to function as it should, at least in the first few days of life. One could argue even that an increased stimulation of the AVP/copeptin system at birth is favorable for preventing dehydration in early neonatal life. Indeed, delayed voiding has been shown to be a function of high AVP levels at birth [64,65].

Besides the effect of AVP on water balance, recent findings have indicated a central role of AVP-receptor signaling in peripheral analgesia [66]. It was found that infants born by vaginal delivery have a natural analgesia within the first hours of life compared to those born by elective C-section [67]. Keeping the here presented high levels of copeptin after vaginal delivery and the AVP-receptor signaling in peripheral analgesia in mind, one may arrive to the conclusion that an AVP/copeptin release at birth is mediating this [60]. Until now many lecturers have accredited oxytocin a central role in birth analgesia, but AVP not oxytocin is increased at birth and it so appears that the AVP receptor is signaling peripheral analgesia [60].

Respiratory morbidity is less frequent after C-section with prior onset of labor compared to after elective C-section, and even less frequent after vaginal delivery [68]. The findings in this study showed a similar pattern, where higher copeptin values were found more frequently after C-section with prior onset of labor compared to after elective C-section, and even more frequently after vaginal birth. Therefore, adequately high AVP/copeptin concentrations at birth may provide a natural advantage in early pulmonary adaptation. In fact, activation of vasopressin receptors situated on type II pneumocytes and pulmonary vasculature endothelium results in surfactant secretion [69] and pulmonary vasodilation, especially under hypoxic conditions [70,71].

Lastly, researchers now widely acknowledge an involvement of brain-located AVP in the modulation of complex social behaviors, including maternal behavior and bonding

[72]. Of course the effects on behavior vary from species to species [73]. In rats, it has been demonstrated that increased AVP activity in certain areas of the brain promotes maternal care [74] and is characteristic of increased maternal aggression [75]. Moreover, AVP is necessary in regulating selective partner preference in adult pair bond formation in prairie voles [76,77], although it remains unclear whether it prompts pair bonding between a mother and her offspring. Recent studies have begun to investigate a similar AVP-involvement also in human behavior [78–81]; it is tempting to hypothesize that the surge of AVP/copeptin after normal delivery is favorable in early mother-child bonding. Nonetheless, queries concerning the interaction of peripheral and central AVP action remain unsolved: first, does AVP found in the circulation cross the blood-brain barrier at birth, even if only at very high concentrations? Second, if the blood-brain barrier is usually tight for AVP, does high AVP affect its permeability? Third, is there an increased neuronal AVP release in the brain in response to stress, for example vaginal delivery? The literature is surprisingly empty especially when searching for human data. Animal models may provide some of these answers, although caution in interpretation is appropriate [60].

In conclusion, the results from copeptin research on adults praise its potential clinical use as a possible diagnostic and prognostic biomarker for illnesses ranging from cardiopulmonary disease to acute life-threatening situations to stroke [47]. One could only expect to find a similar applicability in pediatric medicine. For example, in this study's cohort no asphyxia was present, but colleagues in Bern have recently demonstrated that copeptin levels are consistently very high in cord blood drawn from asphyxiated infants at birth [82]. With that in mind, copeptin should be considered as a potential marker for birth stress and particularly as a possible diagnostic tool for perinatal asphyxia.

6. Bibliography

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